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Highly Potent Synthetic Polyamides, Bisdistamycins, and Lexitropsins as Inhibitors of Human Immunodeficiency Virus Type 1 Integrase

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ABSTRACT

Alignment of the available human immunodeficiency virus type 1 (HIV-1) viral DNA termini [U5 and U3 long terminal repeats (LTRs)] shows a high degree of conservation and the presence of a stretch of five or six consecutive adenine and thymine (AT) sequences ~10 nucleotides away from each LTR end. A series of AT-selective minor-groove binders, including distamycin and bisdistamycins, bisnetropsins, novel lexitropsins, and the classic monomeric DNA binders Hoechst 33258, 4′-diamino-2-phenylindole, pentamidine, berenil, spermine, and spermidine, were tested for their inhibitory activities against HIV-1 integrase (IN). Although netropsin, distamycin, and all other monomeric DNA binders showed weak activities in the range of 50–200 μM, some of the polyamides, bisdistamycins, and lexitropsins were remarkably active at nanomolar concentrations. Bisdistamycins were 200 times less potent when the conserved AAAAT stretch present in the U5 LTR was replaced with GGGGG, consistent with the preferred binding of these drugs to AT sequences. DNase I footprinting of the U5 LTR further demonstrated the selectivity of these bisdistamycins for the conserved AT sequence. The tested compounds were more potent in Mg^{+2} than in Mn^{+2} and inhibited IN^{50-212} deletion mutant in disintegration assays and the formation of IN/DNA complexes. The lexitropsins also were active against HIV-2 IN. Some of the synthetic polyamides exhibited significant antiviral activity. Taken together, these data suggest that selective targeting of the U5 and U3 ends of the HIV-1 LTRs can inhibit IN function. Polyamides might represent new leads for the development of antiviral agents against acquired immune deficiency syndrome.

The rapid emergence of HIV strains resistant to available drugs (Arts and Wainberg, 1996; De Clercq, 1996; Erickson and Burt, 1996) implies that effective treatment modalities will require the use of a combination of drugs targeting different sites of the HIV life cycle (Schinazi, 1991; Johnson, 1994; De Clercq, 1995; Larder *et al.*, 1995). As part of a program to develop novel antiviral agents, we sought to determine the role of specific DNA binding agents as possible inhibitors of HIV-1 IN. IN is an important target for intervention by chemotherapeutics, and to date several inhibitors of this enzyme have been reported (for recent reviews, see Neamati *et al.*, 1997c; Pommier *et al.*, 1997). IN is responsible for the insertion of the viral DNA into a host chromosome. This process is essential for effective viral replication and can be reproduced *in vitro* using recombinant IN and short oligonucleotides (Katz and Skalka, 1994; Rice *et al.*, 1996). Integration takes place in two consecutive steps. Initially, IN processes the linear viral DNA by removing two nucleotides from each 3′-end, leaving the recessed 3′-OH termini. This reaction is followed by transesterification of phosphodiester bonds in which each processed viral 3′ terminus becomes linked to a 5′-phosphate of host DNA strand. These two steps, known as 3′-processing and 3′-end joining (strand transfer), can be easily measured in an *in vitro* assay using purified recombinant HIV-1 IN and an oligonucleotide corresponding to the U5 region of HIV LTR sequence.

IN binds to the viral DNA sequences located at both extremities of the LTRs. Because these sequences are highly conserved in all HIV genomes (Fig. 1), they could provide potential targets for the selective inhibition of integration. In addition, both the U5 and U3 LTRs contain a conserved AT-rich sequence ~10 base pairs from the viral ends (Fig. 1). Netropsin and distamycin (Fig. 2) bind tightly to AT sequences of B-DNA with little affinity for single-stranded nucleic acids, double-stranded RNA, or DNA/RNA hybrids.

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ABBREVIATIONS: HIV-1, human immunodeficiency virus type-1; IN, integrase; LTR, long terminal repeat; ES, electrospray ionization; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TLC, thin layer chromatography, DMF, dimethylformamide; MES, 3-(*N*-morpholino)propanesulfonic acid.

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These molecules have an intrinsic twist that favors insertion into the minor groove of B-DNA (Zimmer *et al.*, 1977, 1983; Kopka *et al.*, 1985; Kopka and Larsen, 1992).

In a search for selective DNA binders with modified properties, novel lexitropsins were designed and synthesized (Lown, 1988, 1994; Lown *et al.*, 1989). Recent studies demonstrated that polyamides can be synthesized to achieve highly selective recognition of the four Watson-Crick base pairs in the DNA minor groove (Helene, 1998; White *et al.*, 1998). In addition, polymethylene-linked lexitropsins were shown to exhibit antiviral activity (Lown *et al.*, 1989; Wang and Lown, 1992). However, their antiviral mechanism is not well understood.

The current report stems from our continuing efforts to identify novel IN inhibitors from compounds that exhibit antiviral activity in cellular assays. Herein, we show that synthetic polyamides that interact selectively with the conserved AT stretch present in the HIV LTRs can inhibit integrations at submicromolar concentrations.

Materials and Methods

Chemistry. All chemicals used were of reagent grade. The reactions were carried out in anhydrous tetrahydrofuran that was dried over sodium/benzophenone and distilled fresh at the time of reaction. Dimethylformamide and triethylamine were distilled and stored over molecular sieves (4 Å). The progress of the reaction was monitored by analytical TLC using silica gel (60F-254 mesh; Merck Research Labs, West Point, PA)-coated aluminum-backed plates. Preparative separations were performed by column chromatography on flash silica gel (70–230 mesh; Merck). Melting points were deter-

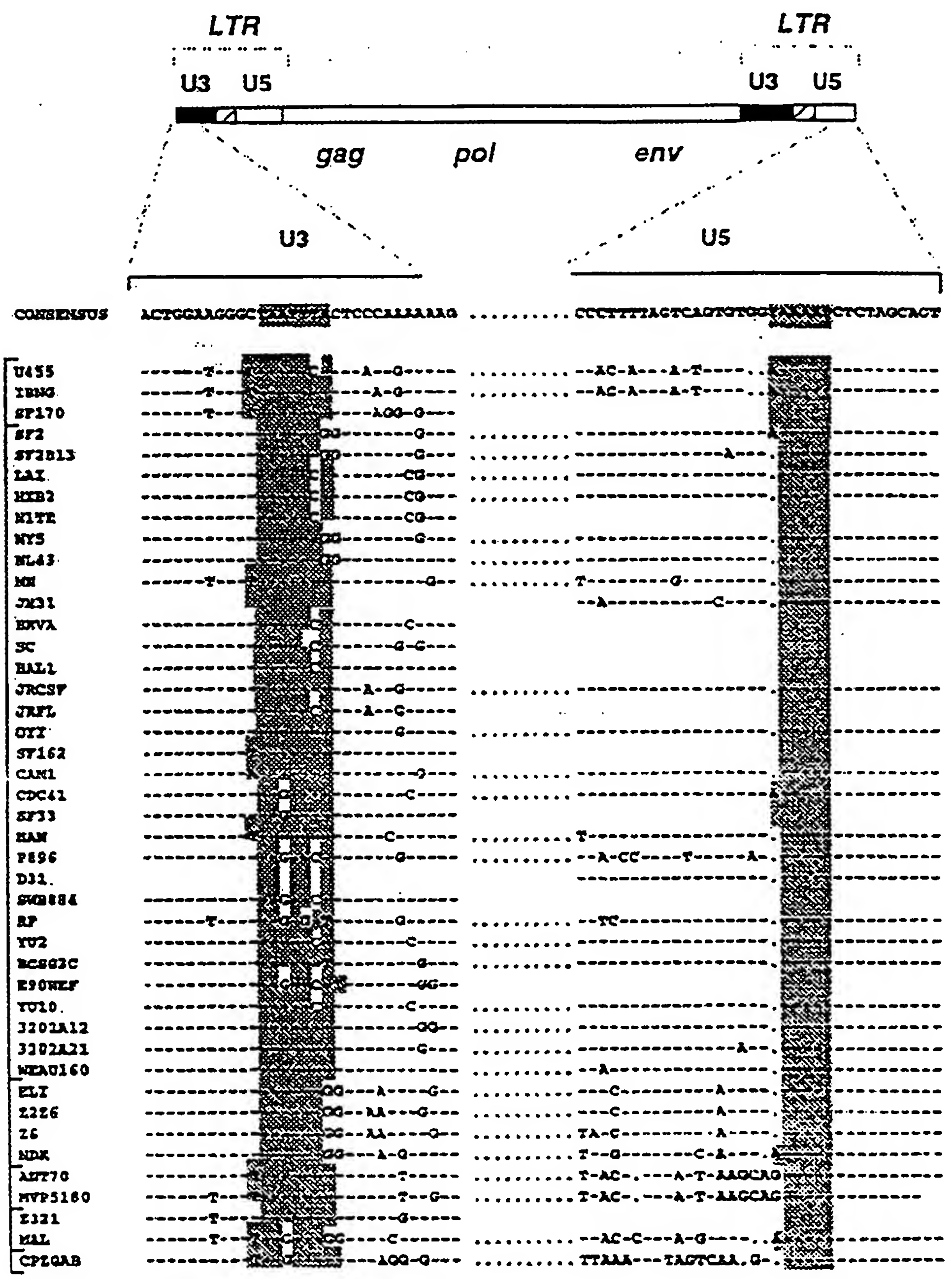


Fig. 1. Nucleotide sequence alignment of the LTR U3 and U5 regions of 43 HIV-1 strains. *Top*, HIV-1 provirus with the LTRs and the three genes *gag*, *pol*, and *env*. *Dashes*, identity. *Periods*, gaps in the nucleotide sequence. *Shaded region*, location of the conserved AT sequence. The sequences were obtained from the Los Alamos HIV Sequence Database Web site (*http://hiv-web.lanl.gov/*).

mined on an electrothermal melting point apparatus (Fisher-John) and are uncorrected. [1]H NMR spectra were recorded on a Bruker AM-300 spectrometer, the samples were prepared in dimethylsulfoxide-d6 unless otherwise specified, and the chemical shifts were reported in δ ppm with respect to tetramethylsilane as an internal standard. New products were characterized by elemental analysis, mass spectroscopic analysis, or both using ES on a Micromass Zabspec Hybrid Sector TOF.

Representative synthesis: *N,N'*-di[1-methyl-2-[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrole)-carboxamido-4-pyrrole]carboxamido-4-pyrrolyl]-phenyl-1,4-dicarboxamide (26). Nitrodistamycin **7a** (0.3 g, 0.6 mmol) was taken in DMF and methanol (10 ml, 1:1, v/v/v) and hydrogenated using Pd-C (10%, 150 mg) on a Parr shaker apparatus at 40 psi for 2 hr at 22°. The catalyst was removed by filtration and washed with methanol. The filtrate was evaporated, and the contents were dried under high vacuum to remove the traces of the solvent. The amindistamycin **7b**, so obtained, was redissolved in anhydrous DMF (4 ml), the solution cooled down to 0°, and 1,4-phenyl diacid dichloride (61 mg, 0.3 mmol), predissolved in anhydrous tetrahydrofuran (4 ml), was added to it followed by 0.5 ml of triethylamine. The reaction mixture was stirred for 2 hr at 22°, at which time the TLC showed complete consumption of **7b**. The solvent was removed *in vacuo*, and the crude product was purified on a silica gel column using CH_2Cl_2/MeOH/NH_4OH (7:3:0.1, v/v/v) as eluent to collect pure **26** (160 mg, 50%); melting point >300° [[1]H NMR δ 1.60 [p, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-$N(CH_3)_2$], 2.16 [s, 12 H, 2 CH_2-$N(CH_3)_2$], 2.28 [t, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-$N(CH_3)_2$], 3.18 [dt, J = 6.0 Hz, J = 7.0 Hz, 2 CH_2-CH_2-CH_2-$N(CH_3)_2$], 3.78, 3.86, and 3.90 (3 s, 18 H, each for 6 protons of pyrrolyl N—CH_3), 6.84, 7.04, 7.14, 7.20, 7.25, and 7.38 (6 d, J = 2.0 Hz, 12 H, each for 2H of pyrrole ring), 8.08 (s, 4 H, phenyl protons), 8.09 (t, J = 6.0 Hz, two CONH-CH_2, merged with phenylic protons), 9.9, 10.02, and 10.50 (3 s, 6 H, each s for two pyrrolyl NHCO); ES[+] calc. for $C_{54}H_{66}N_{16}O_8$, 1067.18; found 1067.50 (M[+], 100%)].

Other bisdistamycins were synthesized in a similar way, and the related data are described below.

***N,N'*-Di[1-methyl-2-[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrole)-carboxamido-4-pyrrole]carboxamido-4-pyrrolyl]-phenyl-1,3-dicarboxamide (27).** This product was isolated in same way as **26**. Yield 225 mg (70%); melting point 295° [[1]H NMR δ 1.64 [p, J = 7.0 Hz, 4 H, two CH_2-CH_2-CH_2-$N(CH_3)_2$], 2.16 [s, 12 H, 2 $N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-$N(CH_3)_2$], 3.20 [dt, J = 6.0 Hz, J = 7.0 Hz, 2 CH_2-CH_2-CH_2-$N(CH_3)_2$], 3.80, 3.88, and 3.94 (3 s, 18 H, each for 6 protons of pyrrolyl N-CH_3), 6.86, 7.06, 7.14, 7.20, 7.30, and 7.40 (6 d, J = 2.0 Hz, 12 H, each for 2 H of pyrrole ring), 7.70 (t, J = 7.5 Hz, 1 H, phenylic H-5), 8.10 (m, 4 H, phenylic H-4 and H-5 merged with 2 CONH-CH_2 protons), 8.50 (s, 1 H, phenyl H-2), 9.90, 10.02, and 10.54 (3 s, 6 H, each s for 2 pyrrolyl NHCO); ES[+] calc. for $C_{54}H_{66}N_{16}O_8$, 1067.18; found 1067.60 (M[+], 100%)].

2*N*-[1-Methyl-2-[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrole)-carboxamido-4-pyrrole]carboxamido-4-pyrrolyl]-2-carboxy benzamide (28). The reaction of **7b** (285 mg, 0.6 mmol) with 1,2-phenyl diacid chloride (50 mg, 0.3 mmol) in the presence of triethylamine (0.1 ml) using DMF as a solvent afforded this product at a higher than anticipated R_f value. Raising the temperature of the reaction mixture to 65° did not result in any change in the nature of the reaction, and some unconsumed **7b** always existed in the reaction mixture (detected on TLC plate). The solvent was removed *in vacuo*, and the crude product was purified on a silica gel column using CH_2Cl_2/MeOH/NH_4OH (7:3:0.1, v/v/v) as eluent to afford 100 mg (54%) of pure **28**. melting point 173° [[1]H NMR δ 1.80 [p, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-$N(CH_3)_2$], 2.48 [s, 6 H, $N(CH_3)_2$], 2.68 [t, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-$N(CH_3)_2$], 3.24 [dt, J = 6.0 Hz, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-$N(CH_3)_2$], 3.80, 3.85, and 3.90 (3 s, 9H, each for 3 pyrrolyl NCH_3), 6.86, 6.98, 7.05, 7.20, 7.26, and 7.30 (6 d, J = 2.0 Hz, 6H, each for 2 H of pyrrole ring), 7.50 (m, 2 H, phenylic H-4 and H-5), 7.65 (dd, J = 7.0 Hz, J = 2.0 Hz, 1 H, phenylic H-6), 7.75 (dd, J = 7.0 Hz, J = 2.0 Hz, 1 H, phenylic H-3), 8.15 (t, J = 6.0 Hz, CONH-CH_2), 9.90, and 10.00 (2 s, 2 H, each s for 2 pyrrolyl NHCO); 11.50 (broad s, 1 H, COOH); ES[+] calc. for $C_{31}H_{38}N_8O_6$ 616.65, found 617.20 (M[+] 100%)].

***N*-[1-Methyl-2-[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrole)-carboxamido-4-pyrrole]carboxamido-4-pyrrolyl]-phthalimide (29).** Phthalic acid (50 mg, 0.3 mmol) and 1,1'-carbonyldiimidazole (100 mg, 0.6 mmol) were heated at 80° in anhydrous DMF and CH_3CN (3:1, 3.0 ml, v/v/v) for 2 hr and cooled to 0°. Distamycin **7b** (285 mg, 0.6 mmol), predissolved in anhydrous DMF (2.0 ml), was added to this reaction mixture, and the contents were stirred for 2 hr at 22°. A TLC examination at this time showed complete disappearance of aminodistamycin and formation of a new product. The solvent was evaporated *in vacuo*, and the crude product was purified on a silica gel column

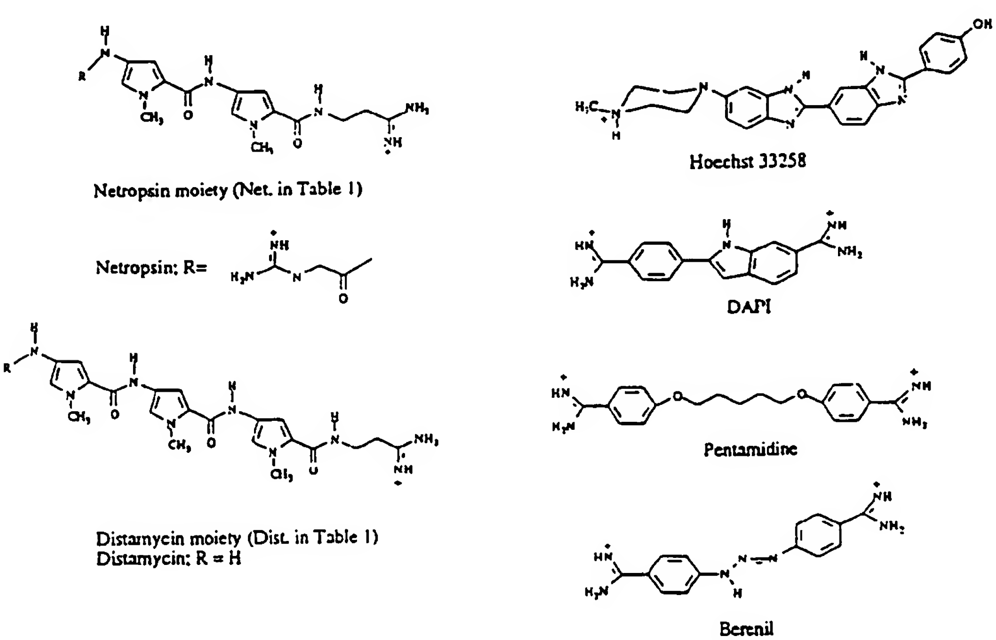


Fig. 2. Structures of minor-groove binders netropsin, distamycin, Hoechst 33258, 4'-diamino-2-phenylindole, pentamidine, and berenil.

using CH_2Cl_2/MeOH/NH_4OH (7:3:0.05) as eluent to give pure 29. Yield 80 mg (44%); melting point 220° [^{1}H NMR δ 1.60 [p, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-N(CH_3)$_2$], 2.10 [s, 6 H, N(CH_3)$_2$], 2.25 (t, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.20 [dt, J = 6.0 Hz, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.80, 3.88, and 3.98 (3 s, 9 H, each for 3 pyrrolyl NCH_3), 6.82, 7.08, 7.20, 7.30, 7.34, and 7.38 (6 d, J = 2.0 Hz, 6 H, each for one pyrrolyl CH), 7.88–7.95 (m, 4 H, aromatic), 8.10 (t, J = 6.0 Hz, CONH-CH_2), 9.90 and 10.10 (2 s, 2 H, each s for 2 pyrrolyl NHCO); ES$^+$ calc. for $C_{31}H_{34}N_8O_5$ 598.63, found 599.10 (M^+ 100%)].

***N,N'*-Di[1-methyl-2-[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrole)-carboxamido-4-pyrrole-]carboxamido-4-pyrrolyl]-pyridine-2,5-dicarboxamide (30).** Reaction of 2,5-pyridine dicarboxy dichloride (61 mg, 0.3 mmol) with amino distamycin 7b (285 mg, 0.6 mmol) in the presence of triethylamine (0.5 ml), using anhydrous DMF as a solvent, gave the crude product. This material was purified the same way as described for 26. Yield 270 mg (85%); melting point >300° [^{1}H NMR δ 1.60 [p, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 2.14 [s, 12 H, 2 N(CH_3)$_2$], 2.25 [t, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.20 [dt, J = 6.0 Hz, J = 7.0 Hz, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.78 and 3.85 (2 s, 12 H, each for 6 protons of pyrrolyl N-CH_3), 3.90 and 3.92 (2 s, 6 H, each for 3 protons of pyrrolyl N-CH_3), 6.84, 7.05, 7.12, 7.19, 7.26, 7.90, and 7.95 (7 d, J = 2.0 Hz, total 10 H of pyrrole ring) 8.10 (t, J = 6.0 Hz, 2 H of 2 CONH-CH_2), 8.25 (d, J = 7.0 Hz, 1 H, pyridyl H-3), 8.50 (dd, J = 7.0 Hz, J = 2.0 Hz, 1 H, pyridyl H-4), 9.20 (d, J = 2.0 Hz, 1 H, pyridyl H-6), 9.90 and 10.00 (2 s, 4 H, each for 2 NHCO), 10.75 and 10.90 (2 s, 2 H, each for 1 H of NHCO); ES$^+$ calc. for $C_{53}H_{65}N_{17}O_8$ 1068.17, found 1068.50 (M^+ 100%)].

***N,N'*-Di[1-methyl-2-[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrole)-carboxamido-4-pyrrole-]carboxamido-4-pyrrolyl]-pyridine-2,4-dicarboxamide (31).** This product was obtained following the synthetic procedure as described for 26. The chromatographic purification, using CH_2Cl_2/MeOH/NH_4OH (7:3:0.05, v/v) as eluent afforded 240 mg (75%) of pure 31 as yellow solid; melting point 200° [^{1}H NMR δ 1.60 [p, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 2.18 [s, 12 H, 2 N(CH_3)$_2$], 2.26 [t, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.20 [dt, J = 6.0 Hz, J = 7.0 Hz, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.80, 3.86, 3.89, and 3.91 (4 s, 24 H, each for 6 protons of pyrrolyl N-CH_3), 6.84, 7.06, 7.16, 7.18, 7.26, 7.42, and 7.44 (7 d, J = 2.0 Hz, total 12 H of 6 pyrrole rings) 8.10 (m, 3 H; 2 H of 2 CONH-CH_2 and 1 H of pyridyl H-5), 8.62 (d, J = 1.5 Hz, 1 H, pyridyl H-3), 8.90 (d, J = 5.5 Hz, 1 H, pyridyl H-6), 9.90 and 10.02, 10.88, and 10.91 (4 s, 4 H, 1 H for each NHCO); ES$^+$ calc. for $C_{53}H_{65}N_{17}O_8$ 1068.17, found 1068.30 (M^+ 100%)].

***N,N'*-Di[1-methyl-2-[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrole)-carboxamido-4-pyrrole-]carboxamido-4-pyrrolyl]-pyridine-2,6-dicarboxamide (32).** The crude mixture was purified on a silica gel column using CH_2Cl_2: MeOH:NH_4OH (7:3:0.05, v/v/v) to obtain 260 mg (81%); melting point 228–30° [^{1}H NMR δ 1.65 [p, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 2.25 [s, 12 H, 2 N(CH_3)$_2$], 2.40 (t, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.20 [dt, J = 6.0 Hz, J = 7.0 Hz, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.80, 3.88 and 3.96 [3 s, 18H, each for 6 protons of pyrrolyl N-CH_3), 6.86, 7.10, 7.18, 7.25, 7.30 and 7.45 (6 d, J = 2.0 Hz, 12 H, each for 2 H of pyrrole ring), 8.08 (t, J = 6.0 Hz, 2 CONH-CH_2), 8.30 (dd, J = 6.5 Hz, 1 H, pyridyl H-4), 8.35 (dd, J = 6.5 Hz, J = 2.0 Hz, 2 H, pyridyl H-3 and H-5), 9.90, 10.10 and 11.10 (3 s, 6H, each s for 2 pyrrolyl NHCO); ES$^+$ calc. for $C_{53}H_{65}N_{17}O_8$, 1068.17; found 1068.50 (M^+, 100%)].

***N,N'*-Di[1-methyl-2-[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrole)-carboxamido-4-pyrrole-]carboxamido-4-pyrrolyl]-pyridine-3,5-dicarboxamide (33).** Yield 241 mg (76%); melting point 210° [^{1}H NMR δ 1.60 [p, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 2.20 [s, 12 H, 2 N(CH_3)$_2$], 2.25 [t, J = 7.0 Hz, 4 H, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.20 [dt, J = 6.0 Hz, J = 7.0 Hz, 2 CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.78, 3.84, and 3.90 (3 s, 18H, each for 6 protons of pyrrolyl N-CH_3), 6.84, 7.06, 7.15 7.20, 7.28, and 7.40 (6 d, J = 2.0 Hz, 12 H, each for 2 H of pyrrole ring), 8.10 (t, J = 6.0 Hz, 2 CONH-CH_2), 8.80 (t, J = 2.0 Hz, 1 H, pyridyl H-4), 9.26 (d, J = 2.0 Hz, 2 H, pyridyl H-2 and H-6), 9.90, 10.05, and 10.78 (3 s, 6 H, each s for 2 pyrrolyl NHCO); ES$^+$ calc. for $C_{53}H_{65}N_{17}O_8$, 1068.17; found 1068.60 (M^+, 100%)].

***N*-[1-Methyl-2-[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrole)-carboxamido-4-pyrrole-]carboxamido-4-pyrrolyl]-4-carboxy pyridine-3-carboxamide (34).** This product was obtained following the procedure described for 28. Yield 95 mg (51%); melting point 192°; [^{1}H NMR δ 1.78 [p, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-N(CH_3)$_2$], 2.48 [s, 6 H, N(CH_3)$_2$], 2.78 [t, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.24 [dt, J = 6.0 Hz, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.80, 3.85 and 3.90 (3 s, 9 H, each for 3 pyrrolyl NCH_3), 6.90, 7.04, 7.06, 7.20, 7.30, and 7.40 (6 d, J = 2.0 Hz, 6 H, each for 1 pyrrolyl CH), 7.46 (d, J = 5.0 Hz, 1 H, pyridyl H-5), 8.12 (t, J = 6.0 Hz, CONH-CH_2), 8.56 (d, J = 5.0 Hz, 1 H, pyridyl H-6), 9.0 (s, 1 H, pyridyl H-2), 9.94 and 10.00 (2 s, 2 H, each for 1 H of NHCO), 13.50 (broad s, 1 H, COOH); ES$^+$ calc. for $C_{30}H_{35}N_9O_6$ 618.64, found 618.64 (M^+ 100%)].

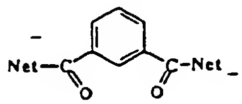
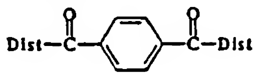
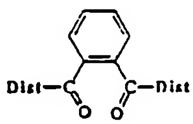
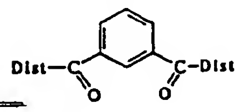
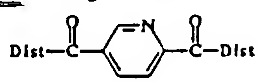
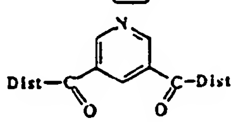
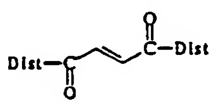
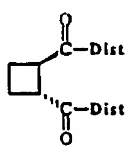
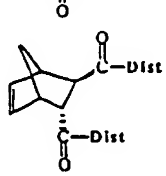
***N*-[1-Methyl-2-[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrole)-carboxamido-4-pyrrole]-carboxamido-4-pyrrolyl]-3-carboxy pyridine-2-carboxamide (35).** This product was obtained following the synthetic procedure as described for 28. Yield 100 mg (54%); melting point 187° [^{1}H NMR δ 1.70 [p, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-N(CH_3)$_2$], 2.30 [s, 6 H, N(CH_3)$_2$], 2.50 (t, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.22 [dt, J = 6.0 Hz, J = 7.0 Hz, 2 H, CH_2-CH_2-CH_2-N(CH_3)$_2$], 3.80, 3.85, and 3.90 (3 s, 9 H, each for 3 pyrrolyl NCH_3), 6.85, 7.06, 7.08, 7.18, 7.25, and 7.32 (6 d, J = 2.0 Hz, 6 H, each for 1 pyrrolyl CH), 7.50 (dd, J = 5.0 Hz, J = 7.5 Hz, 1 H, pyridyl H-5), 8.02 (dd, J = 1.5 Hz, J = 7.5 Hz, 1 H, pyridyl H-4), 8.10 (t, J = 6.0 Hz, 1 H, CONH-CH_2), 8.62 (dd, J = 5.0 Hz, J = 1.5 Hz, 1 H, pyridyl H-6), 9.90 and 10.00 (2 s, 2 H, each for 1 H of NHCO), 10.98 (broad s, 1 H, COOH); ES$^+$ calc. for $C_{30}H_{35}N_9O_6$ 618.64, found 618.10 (M^+ 100%)].

Preparation of radiolabeled DNA substrates, IN proteins and assays, electrophoresis and quantification, and anti-HIV assays in cultured cell lines. These methods were performed essentially as described previously (Neamati *et al.*, 1997b). The Mg^{+2}-based assays were carried in the presence of 5% polyethylene glycol as described previously (Engelman and Craigie, 1995). The anti-HIV drug testing were performed at National Cancer Institute essentially as described by Weislow *et al.* (1989).

Schiff base formation and chemical trapping. IN was incubated with an oligonucleotide-containing an abasic site (see Fig. 7A) in reaction buffer as described above (Mazumder *et al.*, 1996a) for 2 min at room temperature. A freshly prepared solution of sodium borohydride (0.1 M final concentration) was added, and reaction was continued for an additional 5 min. An equal volume (16 μl) of 2× SDS-polyacrylamide gel electrophoresis buffer (100 mM Tris, pH 6.8, 4% 2-mercaptoethanol, 4% SDS, 0.2% bromphenol blue, 20% glycerol) was added to each reaction, and the reaction was heated at 95° for 3 min before loading a 20-μl aliquot on a 12% SDS-polyacrylamide gel. The gel was run at 120 V for 1.5 hr, dried, and exposed in a PhosphorImager cassette. For inhibition of DNA binding experiments, IN (200 nM) was preincubated with the inhibitor (at the indicated concentration) for 30 min at 30° before the subsequent addition of the radiolabeled viral DNA substrate (20 nM) and borohydride. Gels were analyzed using a Molecular Dynamics PhosphorImager (Sunnyvale, CA).

Footprinting experiments. DNase I footprinting was performed in buffer containing 50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin, 10 mM 2-mercaptoethanol, 25 mM MES, pH 7.2, and 10 nM oligonucleotide. Lexitropsins were added to the reaction mixture and incubated at room temperature for 5 min. Digestion was achieved by the addition of DNase I (3 units/ml) for 1 min. The reaction was quenched with EDTA and 2× SDS-polyacrylamide gel electrophoresis buffer (100 mM Tris, pH 6.8, 4% 2-mercap-

TABLE 1
Inhibition of HIV-1 IN catalytic activities and inhibition of HIV-1 replication in CEM cells by a series of polyamides

Compound No.	Structure	IC_{50}		Cellular anti-HIV-1 data		
		3'-Processing	Integration	IC_{50}	EC_{50}	TI
			μM			
1	Net-CO-Net	42.8; 78.4[a]	29.0; 45.0	83.5	11.9	7.01
2	Net-CO$(CH_2)_2$CO-Net	5.8 ± 1.6	7.5 ± 2.5	75.3	12	6.3
3	Net-CO$(CH_2)_9$CO-Net	37.6; 64.8	40.3; 10.0	57	3.9	14.6
4	Net-CO$(CH_2)_{10}$CO-Net	16.4; 33.8	29.2; 10.0	78	6.6	11.7
5		98.9	30.0	148	5.3	28
6		7.5 ± 1.3	6.9 ± 1.4	284	3.55	80
7	Distamycin	56.9	50.2	30.4	NR[c]	
8		0.1 ± 0.08	0.09 ± 0.01	4.7	0.39	12
9		43.7 ± 4.6	10.0			
10		72 ± 64	10.0	140	21	6.6
11		0.08 ± 0.05	0.1 ± 0.03	69	1.6	43
12		33.5	0.4	69	9.8	7.0
13		0.8	0.3	207	10.4	19.8
14		30.7 ± 8.1	10.0	71	16	4.5
15		36.5 ± 4.9	10.0			
16	Dist-CO$(CH_2)_2$CO-Dist	21 ± 8.5	9.5 ± 1.2	41	41	1.0
17	Dist-CO$(CH_2)_6$CO-Dist	0.025; 0.09 (4.9)[b]	0.005; 0.009 (2.2)			
18	Dist-CO$(CH_2)_8$CO-Dist	0.032; 0.09 (7.4)	0.015; 0.09 (6.5)	29	14	2.0
19	Dist-CO$(CH_2)_{22}$CO-Dist	12.2 ± 3.3	8.0			
20	Hoechst 33258	>100	>100			
21	DAPI	>100	>100			
22	Pentamidine	>100	>100			
23	Berenil	>100	>100			
24	Spermine	>100	>100			
25	Spermidine	>100	>100			

[a] Second independent experiment.
[b] Numbers in parantheses refer to GC rich DNA duplex.
[c] NR, not reached.

bis-NetA

effective inhibition. The 1,4 disubstituted *para* derivative 8 exhibited markedly higher potency than the 1,2-*ortho* and 1,3-disubstituted *meta* derivative 9 and 10, respectively. A similar observation was made when the pyridinyl derivative with *para* substitution (compound 11) was compared with the *meta* substituted derivative 12. In addition, the linear 1,2-*trans* substituted compound 13 was 30–50 times more potent than the more rigid cyclobutanyl and norbornyl derivatives 14 and 15, respectively. Compound 12 exhibited remarkable selectivity for the 3'-end joining (strand transfer) step (Fig. 5B). Moreover, the length of the linker also contributes to potency. The dimers with the short dimethylene linker (derivative 16) or a long aliphatic chain linker (derivative 19) exhibited a significantly reduced potency compared with compound 17 or 18 with hexamethylene or octamethylene linkers, respectively.

Novel lexitropsins. A series of novel polyamides also was examined (Table 2). As in the case of the bisdistamycins presented in Table 1, a common structural feature required for potency seems to be the para substitution. For example, the 1,4-disubstituted derivative 26 was markedly more potent than its corresponding 1,3-disubstituted analog, 27. In addition, the 1,4-disubstituted pyridinyl derivative 30 was more potent than its corresponding 1,3-disubstituted derivatives 31, 32, and 33. In accord with the results for the monosubstituted minor-groove binders presented in Table 1, the monosubstituted lexitropsins 28, 34, and 35 were practically inactive. We also found that the novel lexitropsins were active against HIV-2 IN (Table 2).

Classic minor-groove binders. Netropsin and distamycin (Fig. 2) are natural oligopeptide antibiotics with antitumor, antiviral, and antibacterial activities (Zimmer *et al.*, 1977, 1983; Kopka *et al.*, 1985; Kopka and Larsen, 1992). Both antibiotics are known to bind to AT-rich regions in the minor groove of B-DNA in nonintercalative fashion (Zimmer *et al.*, 1977, 1983; Kopka *et al.*, 1985; Kopka and Larsen, 1992). Netropsin, distamycin, Hoechst 33258, 4'-diamino-2-phenylindole, pentamidine, and berenil (Fig. 2), which possess a crescent shape, bind noncovalently in the DNA minor groove without insertion between the base pairs (Zimmer *et al.*, 1977, 1983; Kopka *et al.*, 1985; Kopka and Larsen, 1992). None of these monomeric groove binders (compounds 20-25) exhibited significant activity at 100 μM against IN (Table 1). Distamycin 7 was only weakly active with an IC_{50} value of ~50 μM. Thus, the ability of the polyamides examined to inhibit IN varied considerably. The difference in activity exceeded 2–3 orders of magnitude.

Probing the Mechanism of IN Inhibition

Effect of the polyamides on the HIV-1 IN core region. To examine the mechanism of inhibition of IN, we used an IN deletion mutant, IN^{50-212}, which lacks the amino-terminal zinc-binding region and the carboxyl-terminal DNA-binding domain (Chow *et al.*, 1992; Bushman *et al.*, 1993). This mutant can catalyze an apparent reversal of the integration reaction known as disintegration (Chow *et al.*, 1992) (Fig. 6A). In the disintegration assay, the lexitropsin 26 was markedly more potent than lexitropsin 34 (Fig. 6B). The bisdistamycins 16, 17, 18, and 19 exhibited IC_{50} values of 2.3, 0.009, 0.03, and 3.7 μM (data not shown). The activity of the novel lexitropsins 27-36 in the disintegration assay with IN^{50-212} are indicated in Table 2. These results demonstrate that polyamides can interfere with the activity of the IN core region and that their inhibitory activity does not require the presence of the zinc-binding and carboxyl-terminal domains of IN.

Global nucleophilic inhibition. The 3'-processing reaction involves hydrolysis of a single phosphodiester bond 3' of the conserved CA-3' dinucleotide (Fig. 7A). However, in addition to this hydrolysis reaction, retroviral INs can use glycerol or the hydroxyl group of the viral DNA terminus as the nucleophile in the 3'-processing reaction, yielding a glycerol esterified to the 5'-phosphate, a circular dinucleotide or trinucleotide, respectively (Engelman *et al.*, 1991; Vink *et al.*, 1991; Mazumder *et al.*, 1996b) (Fig. 7A). To examine the effect of synthetic polyamides on the choice of nucleophiles in the 3'-processing reaction, a substrate DNA labeled at the 3'-end with ^{32}P-cordycepin was used (Mazumder *et al.*, 1996b). Compounds 8, 11, 13, and 12 inhibited glycerolysis, hydrolysis, and circular nucleotide formation similarly (Fig. 7B). This result indicates that lexitropsins block indiscriminately all the IN nucleophilic reactions.

Divalent ion effects. Although *in vitro* assays are generally more efficient with Mn^{+2} as a cofactor, it has been proposed that the physiological cation is Mg^{+2}. We compared the extent of 3'-processing and strand transfer for two representative derivatives, 8 and 11, in the presence of Mg^{+2} and Mn^{+2}. Both compounds were more potent with Mg^{+2} than with Mn^{+2} (Fig. 8). This suggests that in contrast to polyhydroxylated aromatics (Fesen *et al.*, 1994; Hazuda *et al.*, 1997b; Neamati *et al.*, 1997a), polyamides do not interact selectively with the divalent metal of the IN catalytic site.

Inhibition of DNA IN binding by polyamides. A recently described DNA IN cross-linking assay (Mazumder *et*

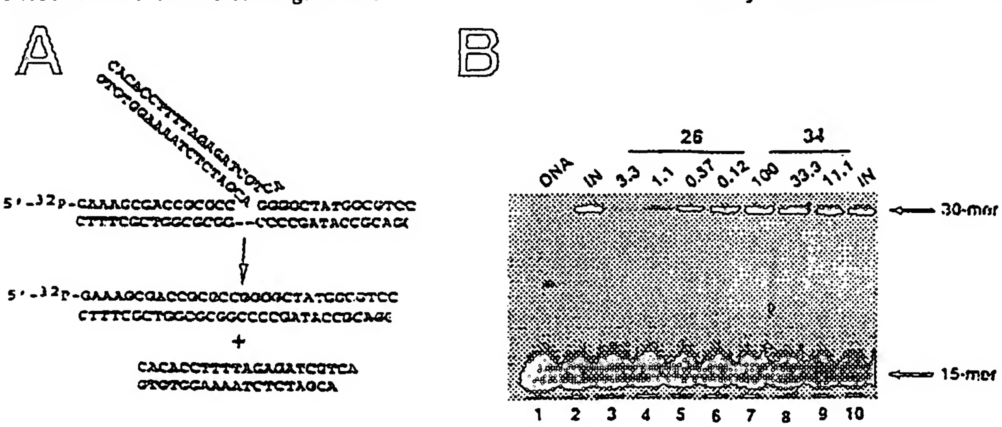


Fig. 6. HIV-1 IN disintegration assay using the core truncated mutant IN^{50-212}. A, The substrate oligonucleotide mimics a strand transfer step product (i.e., a Y oligonucleotide containing a 15-mer oligonucleotide 5'-end-labeled with ^{32}P). HIV-1 IN-mediated disintegration generates a 30-mer oligonucleotide. B, Concentrationdependent inhibition of HIV-1 IN-mediated disintegration by compounds 26 and 34.

ited integration. Second, DNase I footprinting demonstrated the binding of polyamides to the U5 LTR in the conserved AT stretch. Third, changing the AT stretch into a GC stretch reduced the inhibitory activity of the bisdistamycins by 2 orders of magnitude.

The difference in anti-integration potency among the polyamides tested exceeded 2–3 orders of magnitude. This provided a basis for structure-activity relationships. Monomers were relatively inactive even at micromolar concentrations, thus suggesting a requirement of a dimeric form (bislexitropsins) for potent anti-IN activity. Among the bisdistamycins and novel lexitropsins, isomers with *para* substituted aromatic linkers consistently exhibited higher potency than *ortho* or *meta* substituted derivatives (Tables 1 and 2), implying the importance of a linear versus a folded arrangement for activity. Actually, the bisdistamycins with rigid linkers that tended to fold the molecule into a hairpin configuration (compounds **9**, **10**, **14**, and **15**) were not more potent inhibitors than distamycin (compound **7**). The greater activity of the linear bisdistamycins suggests that drug binding to an extended DNA segment is essential for inhibition of integration. Compounds with an aliphatic flexible linker composed of a hexamethylene or an octamethylene chain (compounds **17** and **18**) also were remarkably active, implying the importance of the size of the aliphatic chain linker for optimum potency. Finally, the finding that lexitropsins with substitutions on the distamycin moiety (compare compounds in Tables 1 and 2) remained active inhibitors of IN suggests that further chemical modifications can be made to improve antiviral activity.

In contrast to other IN inhibitors, such as catechol-containing compounds (Hazuda *et al.*, 1997a; Mazumder *et al.*, 1997), the lexitropsins were more active in Mg^{2+}-based assays than in the Mn^{2+}-based assays commonly used *in vitro*. Thus, if Mg^{+2} is more physiologically relevant than Mn^{+2}, it seems that polyamides are potential candidates to block integration *in vivo*.

Targeting of the conserved AT stretches of the LTRs (Bouziane *et al.*, 1996) with lexitropsins (current study) represents a clear strategy for interfering with provirus integration and retrovirus replication. The remarkable conservation of the AT stretches in all the available HIV-1 LTRs (Fig. 1) suggests that synthetic polyamides might generally be active against a broad spectrum of HIV-1 viruses. It is noticeable that some of the synthetic polyamides tested exhibited significant antiviral activity (Table 1). Independent experiments demonstrated that such compounds inhibited reverse transcriptase only at high concentrations (150–200 μM for compounds **1**, **2**, **5**, **6**, **10**, and **16**) (Lown JW, unpublished observations). Such concentrations are much higher than those required to inhibit integration. Further studies are warranted to determine whether integration is a prime mechanism for the antiviral activity of synthetic polyamides and to investigate whether other sites of the HIV genome and other viral processes are targeted by lexitropsins.

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A

5′-^{32}P-GTGTGGAAAATCTCTAGCAGT
CACACCTTTTAGAGATCGTCA

5′-^{32}P-GTGTGGGGGGCTCTAGCAGT
CACACCCCCCGAGATCGTCA

B

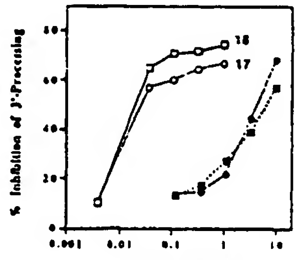

Fig. 10. Importance of the conserved AT sequence for HIV-1 IN inhibition by bisdistamycins. A, The 21-mer blunt-end oligonucleotides where the AT stretch was replaced with GC (*underlined*). B, Concentration-dependent inhibition of HIV-1 IN by compounds 18 (□, ■) and 17 (O, ●) using native 21-mer oligonucleotide (O, ●) or the GC-modified oligonucleotide (■, ●).

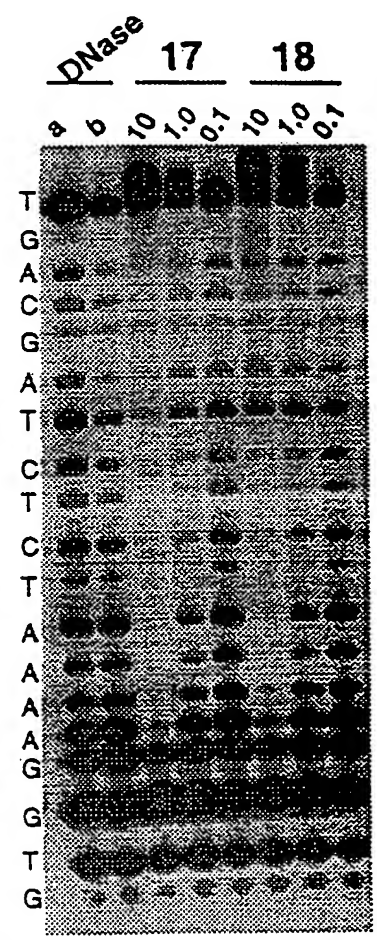

Fig. 11. DNase I footprinting of the 21-mer oligonucleotide corresponding to the U5 end of the HIV-1 proviral DNA, 5′ end-labeled with ^{32}P in the presence of indicated concentrations of lexitropsins **17** and **18**.

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